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Evolutionary lability in floral ontogeny affects pollination biology in Trimezieae

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PREMISE: There is little direct evidence linking floral development and pollination biology in plants. We characterize both aspects in plain and ornamented flowers of Trimezieae (Iridaceae) to investigate how changes in floral ontogeny may affect their interactions with pollinators through time.

METHODS: We examined floral ontogeny in 11 species and documented pollination biology in five species displaying a wide range of floral morphologies. We coded and reconstructed ancestral states of flower types over the tribal phylogeny to estimate the frequency of transition between different floral types.

RESULTS: All Trimezieae flowers are similar in early floral development, but ornamented flowers have additional ontogenetic steps compared with plain flowers, indicating heterochrony. Ornamented flowers have a hinge pollination mechanism (newly described here) and attract more pollinator guilds, while plain flowers offer less variety of resources for a shorter time. Although the ornamented condition is plesiomorphic in this clade, shifts to plain flowers have occurred frequently and abruptly during the past 5 million years, with some subsequent reversals.

CONCLUSIONS: Heterochrony has resulted in labile morphological changes during flower evolution in Trimezieae. Counterintuitively, species with plain flowers, which are endemic to the *campo rupestre*, are derived within the tribe and show a higher specialization than the ornamented species, with the former being visited by pollen-collecting bees only.

KEY WORDS   bee pollination; *campo rupestre*; floral biology; floral traits; flower evolution; heterochrony; nectar; oil flower; paedomorphosis; *Trimezia*.

The role of floral traits in shaping pollination strategies is well documented (Faegri and van der Pijl, 1979; Lloyd and Barrett, 1996; Fenster et al., 2004). These traits, frequently associated with shape, color patterns, scents, timing of anthesis, and especially type of resources, might determine which visitors are attracted to a flower (Fenster et al., 2004; Rosas-Guerrero et al., 2014). During interaction between flower and pollinator, floral traits can affect pollinator behavior and the success of pollen transfer, thus contributing significantly to the reproductive success of a species (e.g., Hansen et al., 2012).

The rate at which floral traits change during the evolution of a group is variable. They may either change slowly (Vasconcelos et al., 2017), the relationships among traits being highly conserved in a phylogenetic time scale (Alcantara et al., 2013), or, alternatively, may be highly labile across the evolution of a group (Smith et al., 2008; Alcantara and Lohmann, 2010, 2011; Smith and Kriebel, 2018).

Rapid evolutionary transformations in floral morphology may result from particular floral developmental processes that lead to abrupt modifications in several floral structures within a short evolutionary time interval (Wagner and Altenberg, 1996; Webster and Zelditch, 2005). These developmental changes have played a major role in the origin and evolution of flowers in angiosperms (Li and Johnston, 2008; Specht and Bartlett, 2009), and specific studies emphasize their role in the evolution of different mating systems (e.g., the evolution of cleistogamous self-pollinating species from outcrossing ancestors; Gallardo et al., 1993; Li and Johnston, 2008). However, surprisingly few studies integrate development with pollination biology to demonstrate how strong variation in floral traits is directly linked to changes in the attraction of floral visitors during the evolution of a group (but see Jaramillo et al., 2004; Armbruster et al., 2012).

The Neotropical tribe Trimezieae in the family Iridaceae provides a useful study case to demonstrate how particular ontogenetic processes may lead to rapid shifts in pollination strategies across the evolution of a group. This clade currently includes five genera, four of which—*Deluciris* A.Gil & Lovo, *Pseudiris* Chukr & A.Gil, *Pseudotrimezia* R.C.Foster, and *Trimezia* Salisb. ex Herb.— are highly endemic to montane, open vegetation habitats, and one, *Neomarica* Sprague, is widely distributed across Neotropical forests. Three of the five genera—*Deluciris*, *Neomarica*, and *Pseudiris*— show typical ornamented iridaceous flowers (Fig. 1: green circles; Table 1; Goldblatt and Manning, 2008). Like other Iridaceae (Hamlin et al., 2017), they are characterized by a showy and conspicuous display, which is mostly colorful, with contrasting color patterns and the presence of glandular trichomes (Table 1; Lovo et al., 2012, 2018). Colors can vary from white to shades of yellow, purple, and blue (Fig. 1), even within the same species, as also observed in other Iridaceae.

However, within both *Pseudotrimezia* and *Trimezia*, although some species possess typical ornamented iridaceous flowers, other species show a contrasting flower display characterized by plain flowers. Plain flowers (Fig. 1: black circles; Table 1) are always yellow, with similar sepal and petal morphologies, always lacking glandular trichomes. Both *Pseudotrimezia* and *Trimezia* include species with both ornamented and plain-flowered species within the same clades, though species with plain flowers are much more frequent in *Pseudotrimezia*. Inference of the ancestral states of individual floral traits related to these morphologies on a well-resolved tribal phylogenetic tree has revealed these flower types to be highly homoplastic (Lovo et al., 2012). Moreover, the entire suite of characters associated with each morphology appears to evolve together. Intermediate floral morphologies are rare, although at least one species within a single subclade of *Trimezia* (*T. martinicensis* [= *T. galaxioides*]; Fig. 1) is not readily classified as either plain or ornamented, presenting instead a mixture of characters of both phenotypes (Fig. 1: red circles; Table 1).

The distribution of the different floral types across the tribal phylogenetic tree (Fig. 1) implies that shifts between plain and ornamented flowers are common in the evolution of the group, especially in the genus *Pseudotrimezia*, and may have led to repeated shifts in pollination strategies across the evolution of this group. However, the underlying processes responsible for such dramatic shifts in overall floral morphology are not well understood. Furthermore, although several studies on the floral biology of Iridaceae have reported distinct floral morphologies connected to various pollination strategies in Paleotropical lineages (Goldblatt and Manning, 1999, 2002, 2006, 2012; Goldblatt et al., 2005; Manning and Goldblatt, 2012; Lavi and Sapir, 2015), information regarding the pollination biology of most Neotropical Iridaceae is relatively sparse (except for the tribe Tigridieae; see Oleques et al., 2020). Combining new data on both floral development and pollination biology in Trimezieae can provide novel evidence for the role of floral ontogenetic changes in the rapid evolutionary shift in pollination strategies.

Here, we investigate floral ontogeny in 11 species of Trimezieae (Fig. 1: blue arrows) and use phylogenetic optimization of floral development to investigate the lability of radical differences in floral morphologies in the evolutionary history of the tribe. Additionally, we employ field observations to investigate how such changes in overall floral morphology could have affected the evolution of pollination strategies in this Neotropical group.

MATERIALS AND METHODS

Taxon sampling and study system

Field expeditions to collect material for micromorphological studies were conducted from 2011 to 2014. Young inflorescences were fixed in FAA and subsequently stored in 70% alcohol. Voucher specimens were deposited in the herbarium of the University of São Paulo (SPF; Thiers, 2019) (Appendix S1). In the present investigation, rather than coding individual mature flower traits that apparently evolved together (Lovo et al., 2012), we investigate the development pathway of overall morphologies in a comparative framework. We define these two different floral morphologies as plain and ornamented, with rare intermediates (Table 1). In addition to color variability, features of the ornamented flowers include clawed sepals and petals, with petals frequently erect, configuring a typical "*Iris*-shape" flower (e.g., *Iris germanica*; Goldblatt and Manning, 2008). The style apex in these flowers also includes several degrees of division, often resulting in highly elaborated petaloid structures (e.g., in *Dietes* Salisb. ex Klatt, *Iris* L., *Moraea* Mill.; Goldblatt and Manning, 2008). By contrast, the plain flowers found in *Pseudotrimezia* and *Trimezia* lack glandular trichomes and striations. Their sepals and petals are morphologically similar and lack claws, the apex of the style is entire, and no other elaborations are observed (Fig. 1).

Micromorphology

Evolutionary change can be investigated by direct comparative observation of the ontogeny of an organ or structure in closely related lineages (Gould, 1977; Guerrant, 1982; Jaramillo et al., 2004; Olson, 2007; Box et al., 2008; Pace et al., 2009; Box and Glover, 2010; Kostyun et al., 2017). This type of comparative study can reveal sources of variation within a group by recording order and rate of organ development, allowing the understanding of structural homologies among different species (Tucker, 1997). We followed this reasoning and investigated floral ontogeny in 11 species of Trimezieae (Fig. 1, blue arrows). Buds were dissected in 70% alcohol under a stereomicroscope, critical-point dried using an Autosamdri-815B critical-point dryer (Tousimis Research, Rockville, Maryland, USA), mounted on aluminium stubs with clear nail polish or carbon disks and coated with platinum in a Quorum Q150T sputter-coater (Quorum Technologies, East Grinsted, UK). Samples were examined and images were taken using a Hitachi S-4700-II (Hitachi High Technologies, Tokyo, Japan) cold field emission scanning electron microscope at the Royal Botanic Gardens, Kew. Flowers at different developmental stages from species representing the entire range of floral morphologies, as well as all the major clades of Trimezieae (Lovo et al., 2012), were investigated to reconstruct their ontogenetic pathway. This sampling method of selecting representatives of the main morphological types with later extrapolation to other closely related species that share these types has been broadly used in comparative ontogenetic studies (Guerrant, 1982; Jaramillo et al., 2004; Box et al., 2008; Armbruster et al., 2012; Kostyun et al., 2017). For a few species, ontogenetic series could not be entirely followed because some development stages were missing and/or flower buds were damaged. Therefore, we present the pathways for three species for which ontogenetic series were complete, representing both floral types (plain and ornamented) and an intermediate phenotype: *Pseudotrimezia pauloi* Chukr (plain flower), *Trimezia martinicensis* (Jacq.) Herb. (intermediate phenotype), and *P. truncata* (Ravenna) Lovo & A.Gil (ornamented
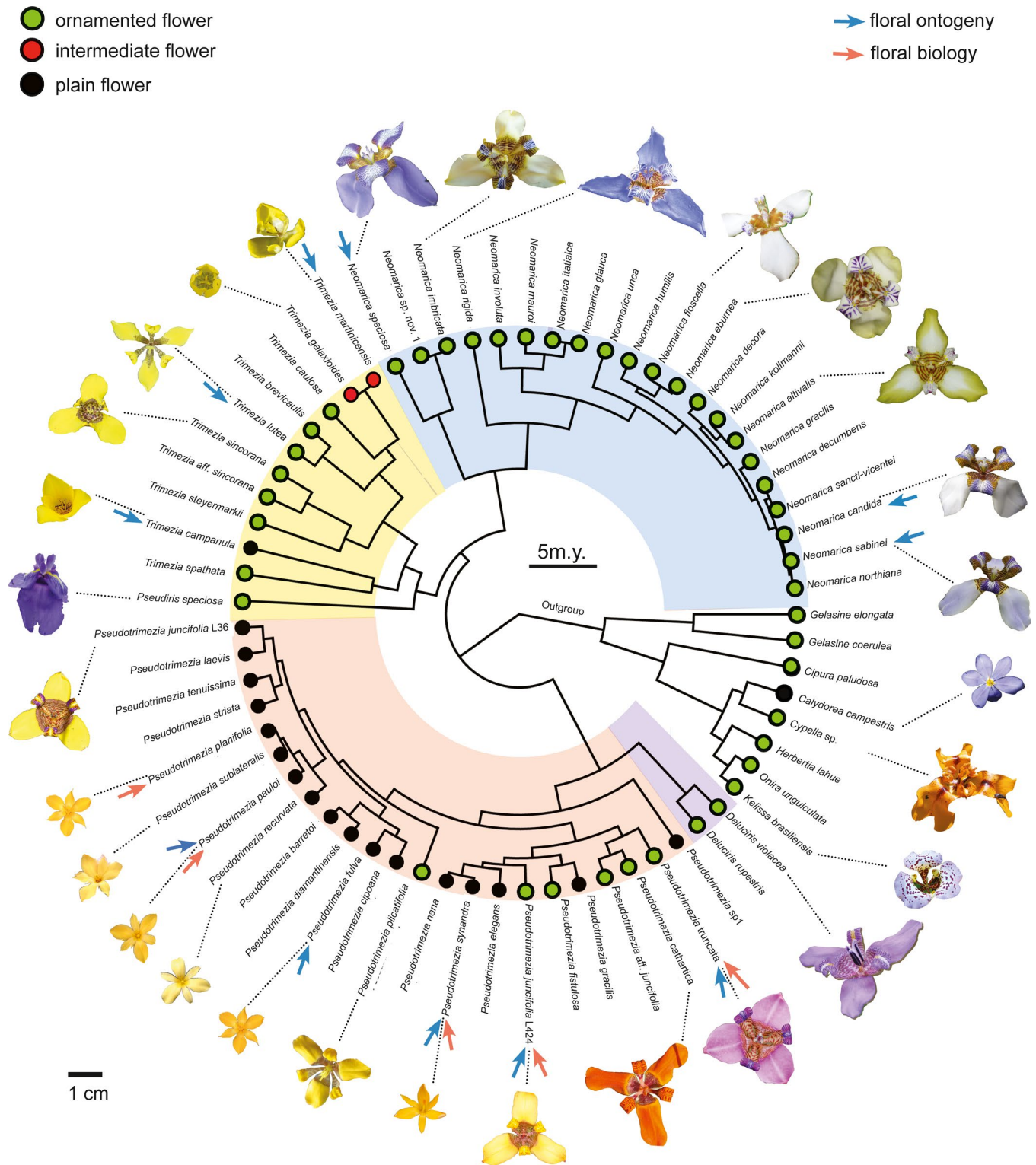
FIGURE 1. Phylogenetic tree of Trimezieae based on combined molecular data from Lovo et al. (2018), showing the five genera examined here and highlighting the homoplastic evolution of different floral types. Blue arrows: species sampled for floral ontogeny; orange arrows: species where pollinator behavior was observed in the field. Black dots: plain flowers; red dots: intermediate flowers; green dots: ornamented flowers. M.y. = million years. Photo credits: A. Conceição—*Pseudiris speciosa*; A. Gil—*Neomarica altivalis, N. candida, N. floscella, N. eburnea, N. imbricata*; G. Heiden—*Cypella* sp., *Kelissa brasiliensis*; J. Lovo—*N. rigida, N. sabinei, N. speciosa, Pseudotrimezia cathartica, P. fulva, P. pauloi, P. planifolia, P. plicatifolia, P. recurvata, P. truncata, Trimezia campanula, T. galaxioides, T. martinicensis, T. sincorana*; L. Temponi—*Calydorea campestris*; R. Mello-Silva—*Pseudotrimezia juncifolia* L424, *P. synandra*; R. Riina—*Pseudotrimezia juncifolia* L36; R. Winkworth—*Deluciris violacea, Pseudotrimezia sublateralis*; S. Alcantara—*T. lutea*).

TABLE 1. Morphological characters of flowers used to delimit and classify the three floral types defined for Trimezieae in this study (modified from Lovo et al., 2012).

Character	Plain	Intermediate	Ornamented
Shape of sepals/petals	Similar[a]	Similar[a]	Different[a]
Ornamentation on petals (color patterning and spots)	Absent/proximal portion speckled	Proximal portion speckled	Proximal portion with transverse bands conspicuous
Glandular trichomes on sepals/petals	Absent	Present	Present
Orientation of petals	Spreading	Ascending	Ascending
Longitudinal position of petals	Planar	Planar	Geniculate revolute
Crests and/or appendages on style apices	Absent	Present	Present

[a]Among themselves.

flower) (Figs. 2–4; Appendices S2 and S3). These species are placed in different major clades in the phylogeny and include the whole spectrum of floral morphological variation in the group (Fig. 1).

Observations of pollinator behavior

To test how each category of floral morphology can affect pollinator behavior, observations of floral visitors were documented in five field expeditions by two of us (J.L and A.J.C.A.) from December 2014 to January 2016 in the Diamantina plateau, central Minas Gerais State, Brazil, one of the centers of diversity in the group (Chukr and Giulietti, 2003; Lovo and Mello-Silva, 2015). As before, we aimed to cover representatives of different morphologies, an approach commonly used in this context (Armbruster et al., 2012). Six populations of five Trimezieae species were observed for recording visitors (Appendices S1 and S4), assigning putative pollinator guilds, describing their behavior, describing floral resources available, and recording time of anthesis, resulting in 60 h of field observation. The species observed, *Pseudotrimezia juncifolia* (Klatt) Lovo & A.Gil, *P. truncata* (Ravenna) Lovo & A.Gil, *P. synandra* Ravenna, *P. planifolia* Ravenna, and *P. pauloi*), represent both plain and ornamented flowers (Table 1; Fig. 1, red arrows). Stigmatic receptivity was checked using hydrogen peroxide, and presence of nectar was recorded using a glycosetape just after floral anthesis. The presence of oil glands were suggested by previous studies (Vogel, 1974, 2009; Buchmann, 1987); therefore, we observed the behavior of oil-collecting bees in the flowers. A few flowers per population were bagged in plastic bags for ≤1 h after anthesis to check the presence of floral scent. Flower visitors were also collected for identification using an entomological net, and the vouchers were deposited in the Entomological Collection of University of Brasília. Field localities and vouchers collected from each population, along with their visitors, can be accessed in Appendices S4 and S5.

Phylogenetic framework

We used the molecular matrix of Lovo et al. (2018) to infer a time-calibrated phylogenetic tree and perform analyses of ancestral state reconstruction of floral development in Trimezieae. We included the same outgroup taxa, consisting of members of Tigrideae (bearing mostly ornamented, but also plain flowers), to root the phylogenetic inference (Appendix S1). The phylogeny presented here is based on the concatenated matrix combined from nuclear and chloroplast data sets from Lovo et al. (2018). The matrix was used to run a phylogenetic analysis based on Bayesian inference in BEAST version 1.8.3 (Drummond et al., 2012). We used a single secondary calibration point in the crown node of Trimezieae as a prior in the analysis, setting it as a mean of 17.4 myr (million years), normal distribution, and standard deviation of 1. This estimated age of divergence corresponds to the mean of the ages found for the same node in two previous studies: Chen et al. (2013) found an estimated age of 11 myr for the most recent common ancestor of Trimezieae, while Goldblatt et al. (2008) estimated 23 myr for the same point in the phylogenetic tree. Considering the confidence intervals and the fact that both ages fall in the Miocene, we assumed that they are not too different to prevent using the mean between them as the best approach (Kumar et al., 2017). The best evolutionary model was estimated a priori using jModelTest version 2 (Darriba et al., 2012) and resulted in GTR+I+G for both partitions. An uncorrelated relaxed clock and a birth-death (incomplete sampling) tree prior were also set as priors in the analysis. MCMC was set for 50 million generations (writing log parameters every 1000) and the analysis was run in Cipres Science Gateway (Miller et al., 2010). Results were checked for convergence and minimum ESS of 200 in Tracer version 1.6.0 (Rambaut et al., 2014). MCC tree was annotated using Tree Annotator version 1.8.3, selecting a burn-in of 10% of the total number of resulting trees (Appendix S6).

Phylogenetic support for the relationships among the clades *Neomarica*, *Pseudotrimezia+Deluciris*, and *Trimezia+Pseudiris*

FIGURE 2. Development of the plain-type flower of *Pseudotrimezia pauloi* Chukr (except panels A and K, which portray the also plain *Pseudotrimezia synandra* Ravenna). (A) Young bud with three sepal primordia. (B) Initiation of stamen primordia opposite the sepal primordia. Ovary sunken in the flower center. (C–D) Young closed bud with sepals, long unicellular trichomes at the apex, and petals and stamens already present at an early stage. (E) Frontal view of opened young flower with sepals (part of one sepal removed) and already initiated stamens and petals. (F) Opened bud with petals removed, showing three carpel primordia and ovary sunken in the center (gynoecium). (G) Flower bud opened at a later stage. (H) Young opened bud, upper view. Stamen and style branches growing together in close contact, the "fitting system" indicated by dashed lines. (I) Young bud opened, sepals removed, showing style growth, slight differentiation of style apices, with lateral growth (green arrows) and fitting system (dashed lines). (J) Detail of three style branches' apices and anthers, both elongated at a later stage of development. Unfused stigmatic region with lateral growth (green arrows) and papillae (dashed lines), and opened margins of style branches (white arrow). (K) Sepal with magnified ventral surface (i) and petal with magnified glabrous ventral surface (ii) and distal (iii) ventral surface. (L) Mature flower of *Pseudotrimezia pauloi*. Abbreviations: A = androecium; Cn = connective; fs = fitting system; G = gynoecium; Hyp = hypanthium; P = petal; Rb = rib; S = sepal; Sg = stigma; St = stamen; Sty = style; Tr = trichomes. Scale bars: 100 μm (A, B); 200 μm (C, D, E, F, G); 500 μm (H, J); 1 mm (I, K); 1 cm (L).
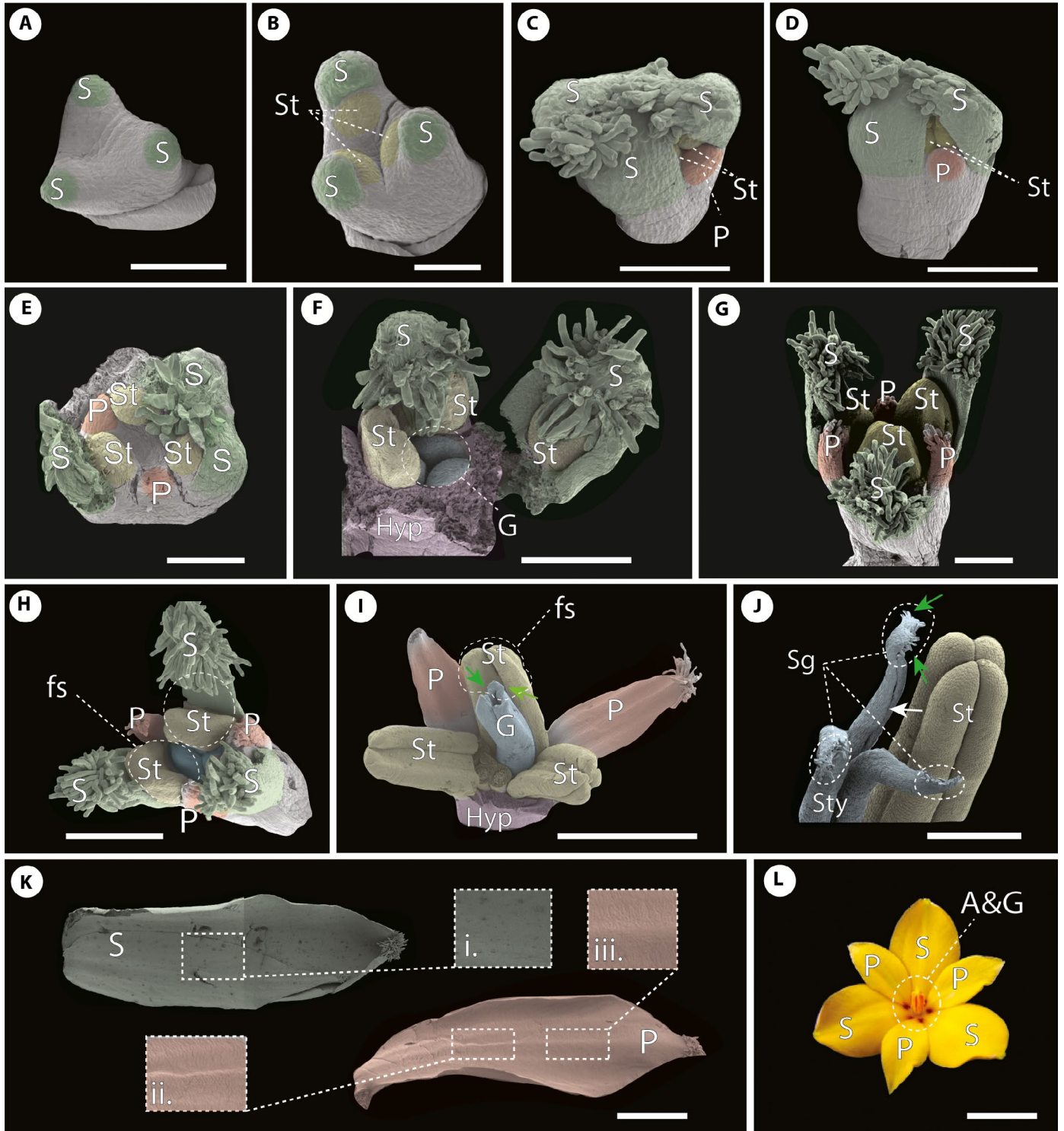
clades is low in the combined tree, probably due to conflict between the nuclear and chloroplast data sets (Lovo et al., 2018). The relationships among genera are not the focus of this study, but, in these circumstances, we inferred dated phylogenies for both nuclear and chloroplast matrices separately, using the same parameters described above and ran all analyses in these alternative trees as well

(see below). For all the further phylogenetic comparative analyses carried out on this study, outgroups were pruned from the phylogeny using the function "drop.tip" from the package "ape" (Paradis and Schliep, 2018) implemented in R (R Core Team, 2018). To avoid multiple accessions per species, species represented by more than one tip were also pruned from the tree before analyses.
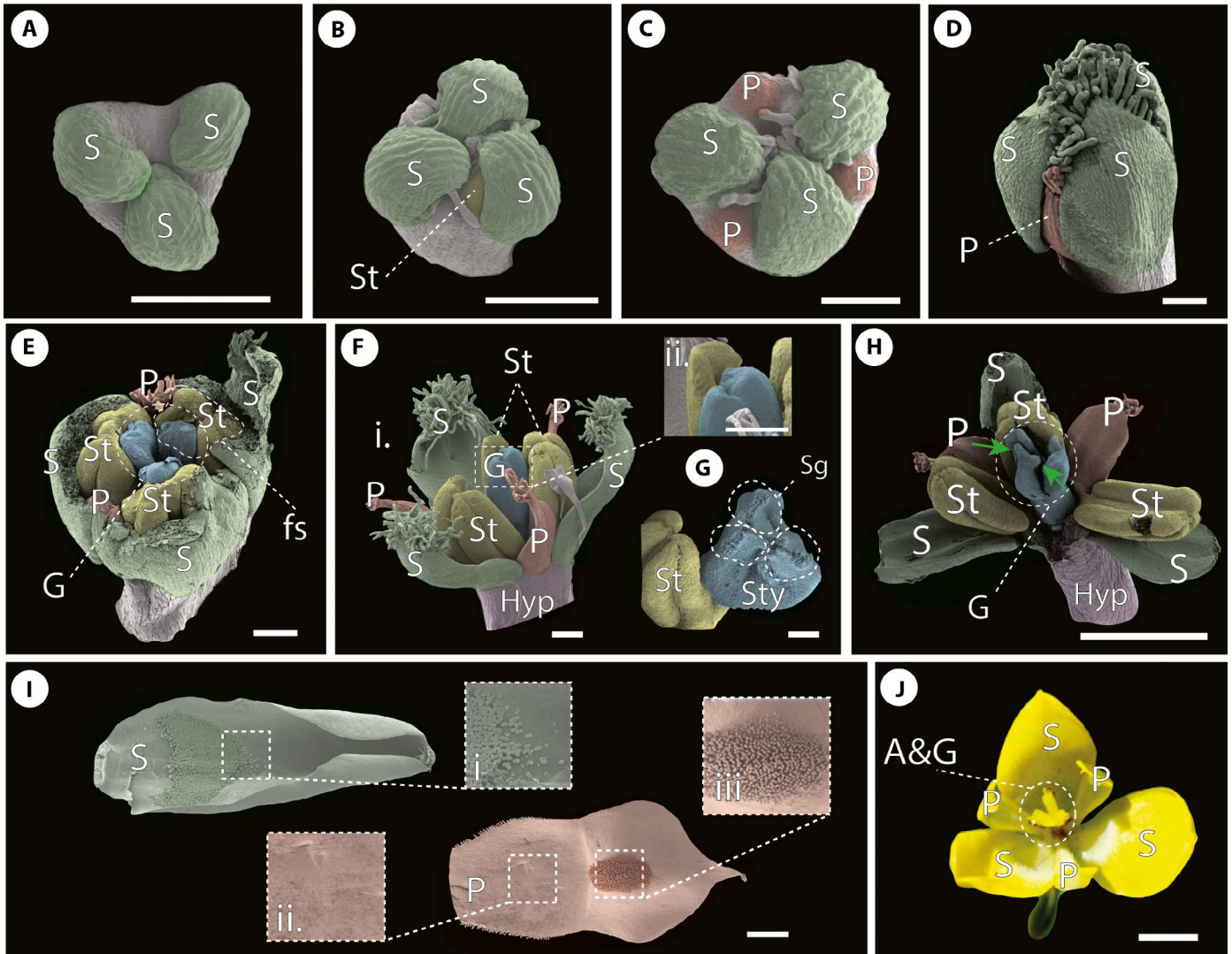
FIGURE 3. Flower development of an intermediate flower of *Trimezia martinicensis* (Jacq.) Herb. (A) Bud with three young sepals incurved toward the center of the flower. (B) Bud with three sepals and stamen formation opposite the sepals. (C) Frontal view of bud closed by the incurved sepals; petal primordia are formed. (D) Lateral view of a closed bud. (E) Bud with organs' already-formed sepals partially removed; detail of the close attachment between style apex and stamen ("fitting system"). (F) (i) Later stage of bud development, opened flower; (ii) detail of the close attachment between anther and style. (G) Upper view of three style apices and one anther partially detached. (H) Bud opened; green arrows indicate inconspicuous lateral growth (wings) of style apex. (I) Sepal with trichomes (i) on the ventral surface and petal with (ii) bottle-shaped trichomes on the proximal region (iii) and also concentrated on the middle region. (J) Mature flower of *Trimezia martinicensis*, showing detail of style apex with three branches (inside dashed circle). Abbreviations: A = androecium; Cn = connective; fs = fitting system; G = gynoecium; Hyp = hypanthium; P = petal; Rb = rib; S = sepal; Sg = stigma; St = stamen; Sty = style; Tr = trichomes. Scale bars: 100 μm (A, B, C, D, E, F, G); 500 μm (H); 1 mm (I); 1 cm (J).

Reconstruction of ancestral flower morphologies

Using our results from floral ontogenetic analyses and additional information from both herbaria and the literature, we coded the floral morphologies of all terminals in the phylogeny into two broad phenotypes plus intermediates: (0) "plain" flowers, (1) "intermediate" flowers, and (2) "ornamented" flowers (Fig. 5; Appendix S1, with codes for all terminals). Each of these floral types is characterized by multiple individual floral traits. Previous analyses have already indicated that these individual traits undergo correlated transitions among character states and, hence, are under common genetic control (Lovo et al., 2012). We also opted for coding a different

category for the species presenting intermediate phenotype (i.e., *Trimezia martinicensis*) in this analysis. In this way, we were able to test whether an intermediate phenotype is required along evolutionary transitions between plain and ornamented types.

To examine inferences about the ancestral floral morphologies in the phylogeny of Trimezieae, we used stochastic character mapping (Bollback, 2006) implemented through the function "make.sim-map" in the R package "phytools" (Revell, 2012; R Core Team, 2018). This method uses a transition matrix between states as a prior, and reconstructs probabilities of ancestral trait states, simulating trait evolution along the branches of the phylogeny. To this end, we first

tested which model of transition matrix best fits our data by comparing AIC_c scores among four models: equal rates ("ER"), all rates different ("ARD"), symmetric ("SYM"), and "ordered" (defined manually including one rate parameter between state "intermediate" and the other two states and a different rate parameter between "plain" and "ornamented" states). The ordered model received lowest AIC_c score (ER = 61.7, SYM = 60.6, ARD = 59.5, and "ordered" = 58.7) and it was used to build the reconstruction in all MCC trees (combined nuclear+chloroplast and in each matrix separately; Appendices S7 and S8). We ran 1000 simulations for each analysis and summarized the results using the function "describe.simmap" from the same package.

RESULTS

Early development

All flowers observed here share similar early ontogeny. Floral development is initiated with three triangular primordia (Fig. 2A, B; Fig. 3A; Fig. 4A); these represent common (complex) primordia that immediately split into two: three sepal primordia outside three stamen primordia (Fig. 2B–E; Fig. 3B). The presence of three stamens located opposite the sepals is typical of Iridaceae, in which only three stamens are present (Goldblatt and Manning, 2008). At initial stages of sepal elongation, uniseriate trichomes start to grow on the apex of each young sepal, while the young sepals extend in a curve toward the center of the flower (Fig. 2C, D; Fig. 3B, C; Fig. 4B). The trichomes grow rapidly in length and soon become almost a third of the size of the sepal (Fig. 2C; Fig. 3C, D; Fig. 4C, D). In the ontogenetic organ sequence, three small petal primordia are initiated, in alternate positions to each sepal (Fig. 2C; Fig. 3C, D; Fig. 4B–D). As each sepal apex touches each other at the top of the bud, they entangle to keep the bud closed (Fig. 2C, D; Fig. 3D). At this point, a shallow depression is present in the center of the flower, forming the inferior ovary (Figs. 2C and 4C). The young stamens continue to grow opposite and in close contact with each sepal. The final organ to develop is the gynoecium, with three distinct carpel primordia located opposite the stamens and sepals (Figs. 2F, 3E, and 4G). These three primordia are initially triangular; they grow in length and start to fold slightly along their middle portion, forming a ventral cleft (Fig. 2I; Fig. 3F; Fig. 4G, H). Their increasing size is associated with postgenital fusion of the three carpels and development of a single style (Figs. 2I, 3H, and 4H). The single style elongates, while the distal portions of the three carpels remain free, forming three style branches (Figs. 2J, 3H, and 4J).

A hypanthium is formed by intercalary growth so that the sepals, petals, and stamens are united at their bases (Figs. 3F and 4H); the growing hypanthium surrounds the style base, leaving a narrow chamber between the hypanthium wall and the style base (Fig. 4J). At this point, the free lateral edges of each style apex grow outward, forming lateral wings (Fig. 2I, J; Fig. 3H; Fig. 4H, J, K). At the same time, each young stamen folds through its axis throughout its entire length, forming two lateral pollen sacs linked by a central connective that can be prominent in some species (Fig. 2I; Fig. 3E, H; Fig. 4H). Each stamen primordium becomes an oblong anther as long as the entire style plus style branches (Fig. 2J; Fig. 3H, J; Fig. 4H).

The stamens appeared earlier in ontogeny, and possibly as a consequence, they elongate in intimate contact with the outer walls of the style, thus forming a delicate and exquisite "fitting system" (Fig. 2H, I; Fig. 3E, F(ii); Fig. 4G, J(ii); Appendix S3F), where each anther connective holds the dorsal surface of the opposite style branch. This contact region of each style branch grows outward, forming a rib (Fig. 2H, J, K; Fig. 3F(ii), G; Fig. 4H, J(i), K). The fitting system is present in all flowers observed, but is more conspicuous in species with ornamented flowers (Fig. 4J, M). As the anthers develop, differential growth continues at the apex of each style branch. Finally, a filament is differentiated by intercalary growth, attached to the hypanthium at its base.

Late development

In all plain flowers investigated, no major differentiation takes place after the organs are initiated; subsequent development is mostly related to growth in length and epidermal differentiation (e.g., papillae, trichomes). Sepals and petals grow in length, with sepals always remaining wider than petals (Fig. 2K, L; Fig. 3I, J; Fig. 4L, M; Appendix S3). The trichomes at their apex become shorter in relation to flower size (Figs. 2K, 3I, and 4L). The three free style branches elongate to half (or even more) of the length of the style. The margins of each style branch tube do not fuse but instead form a free open stigmatic region (Fig. 2J). The cells at the apex of the style branches develop into papillate stigmatic regions (Fig. 2J).

By contrast, in species with ornamented flowers, major changes take place after the shared stages of early ontogeny. Most of these changes are related to the style, sepals, and petals, but sometimes also to the anther. Differentiation of the style apex begins with continuous lateral growth at the margins of the apex, forming more conspicuous lateral wings (Fig. 3H and Fig. 4J, K, green arrows). Almost simultaneously, the dorsal region of each style branch grows outward, resulting in a very prominent curved rib (Fig. 4H, J, K, blue arrows, also indicated by dashed line), perfectly fitting the ridge formed by the anther lobes. The ventral surface of the style branch extends upward, forming two (sometimes more) ventral erect crests (Fig. 4J, K, pink arrows).

During the final stages of development in ornamented flowers, epidermal cells across the style apex increase differentiation and the stigmatic region becomes densely papillate (Fig. 4J, K). A few papillae are also formed on the style apex of the simpler plain flowers, but they lack the densely covered epidermis that characterizes the style apex in ornamented flowers. In ornamented flowers, trichomes are differentiated on the ventral surfaces of the sepals and petals. Two different types of trichome are formed, depending on their position on the organ. On both sepals and petals, proximal cells become rounded and develop into spherical glandular trichomes (Fig. 4L [i, ii]), becoming bottle-shaped toward the center of the organ (Fig. 4L [iii]). The bottle-shaped trichomes follow longitudinal lines along the sepal or petal surface (Fig. 4L).

In the species presenting intermediate phenotype, the ontogenetic pathway follows the same initial steps as in plain flowers, but additional steps are observed during the formation of the apical portion of the style, leading to further elaboration of the style apex. The style becomes divided and slightly different from that of plain flowers, but not as elaborate as those found in ornamented flowers. Sepals and petals also show some ornamentation and only one type of glandular trichome (Fig. 3I [i, iii]) spread over the middle of the perianth surface (Fig. 3I). Mature flowers of the three described types can be seen in greater detail in Appendix S3.
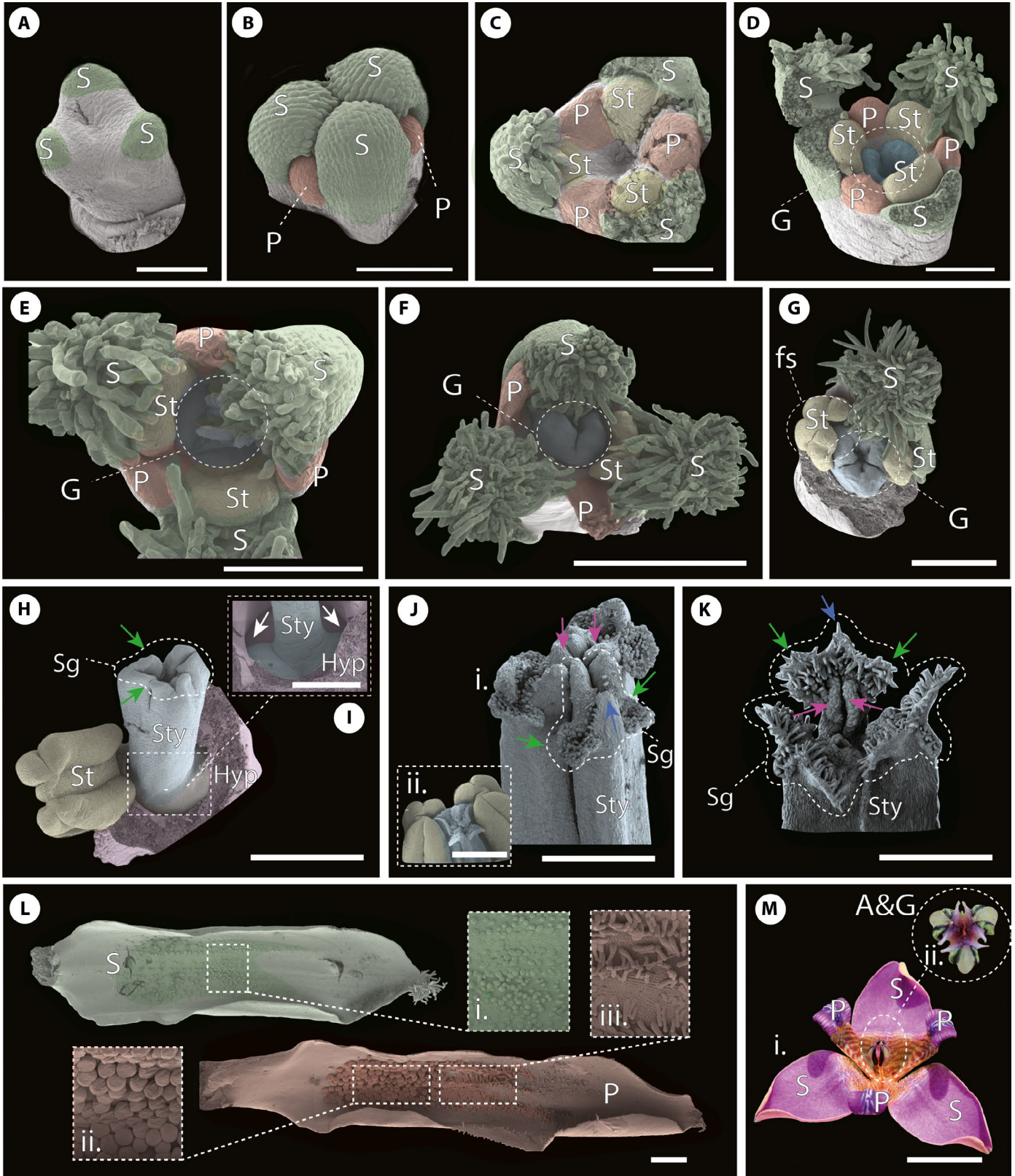
Floral visitors and foraging behavior

Field observations revealed that bees and hoverflies were the most frequent visitors to both plain and ornamented flowers (Table 2).

Other insects also visited, but with lower frequency; they were rarely observed to collect resources or touch the stigmatic region (Table 2).

FIGURE 4. Flower development of an ornamented-type flower of *Pseudotrimezia truncata* (Ravenna) Lovo & A. Gil (except A and I–K, which portray the also ornamented *Pseudorimezia cathartica* (Klatt) Ravenna). (A) Young bud with three sepal primordia. (B) Young bud enclosed by the sepals, petal primordia arising between the sepals. (C) Young bud opened with sepals, petals, and stamen. (D) Young bud opened and two sepals removed showing initiation of all organs, including three carpel primordia in the flower center. (E) Upper view of young opened flower; dashed circle around three carpel primordia forming the gynoecium with inferior ovary. (F) Young bud opened, three carpel primordia in the center, with lateral margin beginning to fuse (highlighted by the dashed circle). (G) Upper view of a young bud, showing the close position of anthers and style branches. (H) Young bud with sepals and petals removed. Fusion of lateral margins of three gynoecium primordia originating a single style. Stigmatic region indicated by dashed line, with three separate style apices. Green arrow indicating initial lateral growth in a wing-like structure. (I) Detail showing the small chamber (white arrow) formed by hypanthium growth around the style. (J) (i) Detail of the apex of three style branches (stigmatic region). Dashed line circumscribing one style apex with specialized developing structures. (ii) Detail showing the "fitting system" formed by the inner contact between dorsal rib of style branch fitting into the filament space. (K) Style apex elaborations: green arrows indicate conspicuous lateral growth; blue arrow indicates dorsal growth forming a prominent dorsal rib at each style branch; pink arrows indicate ventral growth of style apex forming two erected crests. Stigmatic region covered with papillae. (L) Sepals and petals on the ventral surface showing trichomes: (i) details of spherical trichomes on sepal surface; details of petal showing (ii) round-shaped trichomes in the proximal region and (iii) bottle-shape trichomes in the middle to distal region organized in longitudinal lanes. (M) (i) Mature flower of *Trimezia truncata* and (ii) detail of upper view showing three style apexes in the middle and three attached stamens. Abbreviations: A = androecium; Cn = connective; fs = fitting system; G = gynoecium; Hyp = hypanthium; P = petal; Rb = rib; S = sepal; Sg = stigma; St = stamen; Sty = style; Tr = trichomes. Scale bars: 200 μm (A, B, D, E); 100 μm (C); 500 μm (F, G, H, I, J, K); 1 mm (l); 3 cm (m).

In the plain flowers of *Pseudotrimezia pauloi*, *P. planifolia*, and *P. synandra* (Fig. 6B; Appendix S9), visitors flew straight to the central structure in the flower that combines the stamens and style (Fig. 6A, B; Appendix S9). They actively collected pollen by foraging on anthers and touched the stigmatic region in the process (e.g., Fig. 6C, D). Pollen was the sole observed reward; tests for nectar presence, using a yellow stripe that turns into blue in the presence of glycose, showed low to no sugar content (Appendix S9). The three species observed with plain flowers always reached anthesis late in the afternoon, and the period of stigma receptivity (indicated by peroxide test) lasted ~3 h, which is the total time of an opened flower. Visitor activity was intense during this period.

Visitors foraging on the ornamented flowers of *P. juncifolia* (Fig. 6E–H; Appendix S10) and *P. truncata* (Fig. 6I–L; Appendix S11) displayed different behavior. The insects, mostly bees, including some oil-bees, usually landed on the sepals and petals (e.g., Fig. 6G, K; Appendices S9 and S11). Instead of flying straight to the central structure, they foraged over the nectar guide area, which is full of glandular trichomes (Fig. 6F, G, J, K; Appendices S10 and S11). Nectar production was also conspicuous, with high sugar content shown by the glycosetape test (Appendices S10 and S11). After foraging over the ornamented area, some bees moved to the stamens and collected pollen, touching the stigmatic area in the process (Fig. 6H, L; Appendices S10 and S11). Occasionally, the weight of larger-bodied bees foraging the glandular area made the petals fold toward the center of the flower so that their bodies touched the stigmatic area (Fig. 6K; Appendices S10 and S11), showing a hinge mechanism mediating pollen transfer (Appendices S12 and S13).

TABLE 2. Time of anthesis and flower visitors in each Trimezieae species observed in the field.

| Type of flower / species	Flower color	Time of anthesis (length of visiting)	Flower visitors	Resource collected	Size of population (n individuals)
Plain flower					
Pseudotrimezia pauloi Chukr	Yellow	5:30 pm (3 h)	Hymenoptera: *Aughochlorella urania*, *Paratrigona lineata*	Pollen	50
Pseudotrimezia planifolia Ravenna	Yellow	4:30 pm (3 h)	Hymenoptera: *Arhysoceble* sp.1[a]	Pollen	30
Pseudotrimezia synandra Ravenna	Yellow	5:20 pm (2.5 h)	Diptera: Syrphidae *Allographta* sp.	Pollen	60
Ornamented flower					
Pseudotrimezia juncifolia (Klatt) Lovo & A.Gil (yellow flowers)	Yellow	7:00 am (5 h) 12:00–1:00 pm (5 h)	Hymenoptera: *Arhysoceble* sp.1[a], *Augochlora amphitrite*, *Augochlora* sp.1, *Augochlorella urania*, *Dialictus* sp.1, *Dialictus* sp.2, *Paratetrapedia punctata*[a]	Pollen, oil, nectar	
Pseudotrimezia truncata (Ravenna) Lovo & A.Gil (pink flowers)	Pink/purple	1:00 pm (5 h)	Hymenoptera: *Arhysoceble* sp1[a], *Allograpta* sp.1, *Augochlora amphitrite*, *Augochloropsis* sp.1, *Augochloropsis* sp.2, *Augochloropsis* sp.3, *Callonychium* sp. *Dialictus* sp.1 *Dialictus* sp.2 *Trigona fulviventris*	Pollen, oil, nectar	

[a]Oil-collecting bee.
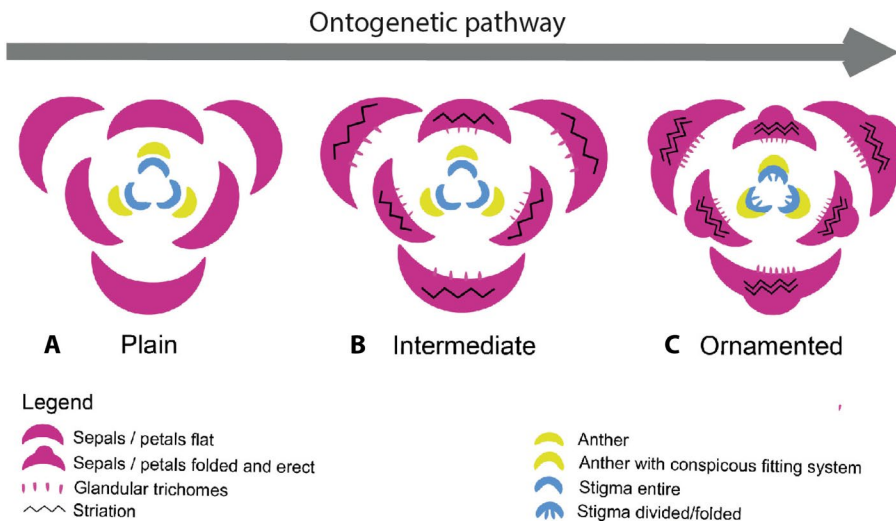
FIGURE 5. Schematic ontogenetic pathway illustrating the three morphological flower types in Trimezieae: (A) plain, (B) intermediate, and (C) ornamented (not to scale).

Phylogenetic inference and ancestral floral morphology

Ancestral floral morphology reconstruction demonstrates the prevalence of ornamented display among the deepest nodes in the tribal phylogenetic tree, with recurrent shifts from ornamented to plain flowers, especially in *Pseudotrimezia* (Fig. 7A, green and black arrows). There were ~10 changes between states across the phylogeny, according to our results from 1000 simulations of character mapping (Fig. 7A). Most of these changes were shifts from ornamented to plain display and vice versa, with one shift observed from ornamented to intermediate (Fig. 7A: red arrow). Most shifts are concentrated within a short time interval of the past 5 myr (Fig. 7A, yellow rectangle). Shifts are equally frequent in both directions, highlighting an evolutionarily labile pattern caused by heterochrony (Fig. 7B). Analyses of floral traits over phylogenies reconstructed from either nuclear or chloroplast matrices separately did not significantly change the results presented here from the combined data set (see Appendices S5 and S6).

DISCUSSION

Floral heterochrony in Trimezieae: accelerating floral evolution by simple ontogenetic steps

Our results show that the two contrasting floral phenotypes of Trimezieae are closely similar during early ontogeny (Figs. 2–4 and 7). However, in the species with plain flowers, development seems to cease at an early stage, possibly indicating paedomorphosis (Garstang, 1922; Gould, 1977). Paedomorphosis represents a type of heterochrony (i.e., temporal change in the expression of a trait between a putative ancestor and its descendants; Gould, 1977; Rudall and Bateman, 2004; Li and Johnston, 2008; Vasconcelos et al., 2017). In this case, it results in a simpler morphology with reduced flowers that resemble a juvenile of the ancestral morphology (Rudall and Bateman, 2002; Olson, 2007; Box and Glover, 2010). The phylogenetic framework allows us to infer that the ornamented morphology was already present in the ancestral lineage of the group. These heterochronic events leading to plain flowers, and sometimes vice versa, appear to be frequent in Trimezieae. They may be especially common in the genus *Pseudotrimezia*, where most of the abrupt transitions between plain and ornamented flowers are observed.

As observed in Trimezieae, heterochrony can lead to rapid changes in flower morphology by altering multiple floral traits simultaneously. The way in which a flower is organized in interconnected modules during ontogeny (Wiltshire et al., 1994; Pigliucci, 2003; Armbruster et al., 2004) means that major changes in morphology can occur by simple steps affecting all organs when heterochrony takes place. This situation is even more prominent in monocots, where sectorial differentiation is incredibly widespread, probably due to the trimerous floral organization (Endress, 1995; Remizowa et al., 2010). Heterochronic events are common and can be the major cause of ontogenetic modifications during the evolution of a group (Gould, 1977; Endress, 1995; Bateman et al., 2006; Li and Johnston, 2008). This has been highlighted as an important mechanism related to the evolution of new floral forms during the diversification of several angiosperm groups, even including the origin of the flower (Laurent et al., 1999; Box et al., 2008; Li and Johnston, 2008; Specht and Bartlett, 2009; Box and Glover, 2010; Kostyun et al., 2017; Thaowetsuwan et al., 2017).

Implications of paedomorphosis to ecological specialization in Trimezieae

Our results showed that the foraging behavior of visitors differed between plain and ornamented flowers, even when the visitors were of the same pollinator guild, demonstrating that floral paedomorphosis can affect the pollination strategy in Trimezieae. A similar connection between paedomorphosis and switch in pollination strategies has already been suggested for different angiosperm lineages (Guerrant, 1982; Laurent et al., 1999; Ojeda et al., 2017). In Trimezieae, profound modification of the development pathway of plain flowers is associated with suppression of floral features of ecological relevance such as glandular trichomes, hinge-like petals, and style apex elaboration. We observed that ornamented flowers are visited by a larger array of pollinator guilds, attracting bee species that do not visit plain flowers. Ornamented flowers commonly present larger displays and have a broader spectrum of visually appealing traits (Faegri and van der Pijl, 1979; Sapir et al., 2006), such as variation in color and nectar guides, and also offer a broader variety of floral resources other than pollen, including nectar and possibly oil (Goldblatt and Manning, 2006, 2008; Chauveau et al., 2012).

Nectar production is associated with a broad spectrum of visitors attracted by these flowers, including larger-bodied insects (Goldblatt and Manning, 2006, 2008). Even though the presence of oil was not directly tested here, previous studies suggest that the bottle-shaped trichomes that arise during late stages of floral development in *Trimezia* may be elaiophores (Vogel, 1974; Buchmann, 1987; Renner and Schaefer, 2010; Silvério et al., 2012). This inference is reinforced by the oil-collecting bees visiting the ornamented
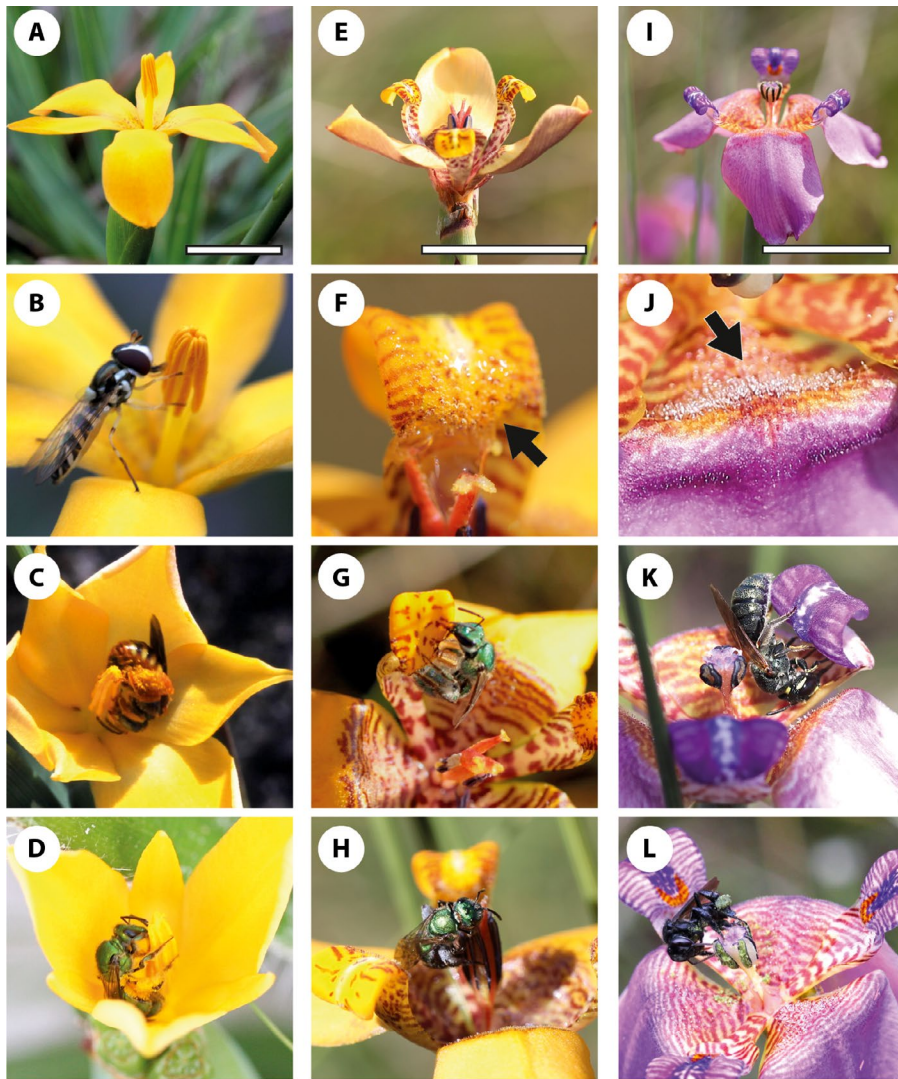
FIGURE 6. Flower visitors and pollination behavior in plain flowers of (A, B) *Pseudotrimezia synandra* Ravenna, (C) *P. planifolia* Ravenna, and (D) *P. pauloi* Chukr; and in ornamented flowers of (E–H) *P. juncifolia* (Klatt) Lovo & A.Gil. and (I–L) *P. truncata* (Ravenna) Lovo & A.Gil. (A, E, I) Flower in anthesis. (B) *Allograpta* sp. (Syrphidae). (C) *Arhysoceble* sp. (Apidae). (D) *Augochlora* sp. (Halictidae) collecting pollen at the beginning of anthesis. (F) Detail on petal with glandular trichomes (black arrow). (G) Sweat bee (Halictidae) on inner sepal imbibing nectar from glandular trichomes and (H) on the column formed by style and anthers, collecting pollen and touching stigmas. (J) Dense glandular trichomatous region on the folding inner tepal (black arrow). (K) *Ceratina* cf. *maculifrons* activating the hinge mechanism. (L) *Trigona* sp. over the column, collecting pollen (corbicula full of green-blue pollen from *P. truncata*). Scale bars: 1.5 cm (A), 2.5 cm (E), 3.5 cm (I). Photo credits: J. Lovo—A, B, C, E, F, H, I, J, K, L; R. Mello-Silva—D; M. Kubo—G. For complete plates, see Appendices S9–S11.

The ornamented flowers phenotype also affects the pollination strategy by modulating visitor interaction during the visit. The striking interaction between flowers and large-bodied visitors, described here as a hinge mechanism, seems to greatly increase the chances of cross pollination by forcing physical contact between the body of the animal and the stigmatic surface. Due to this hinge mechanism of the petals, the visitors attracted by these flowers can occasionally execute pollen transfer even when they are not actively foraging close to the stigma (Appendix S10H; Appendix S11H, I).

Similarly, the specific distribution of glands on the perianth, mostly on the petals, is not random in ornamented flowers and can act as a tactile cue to visitors (Guo, 2015). Similar petal shapes, colors, and guide patterns to those observed here are associated with stamen and pollen mimicry in *Iris* and other angiosperm clades, especially in the alpine flora (Lunau et al., 2017). However, based on the apparent oil secretion of the petal glands, we question whether the term *mimicry* is suitable for this system, since it seems not to be based on a deception mechanism. The wings and papillae on the style apex might maximize the receptive surface of the stigma, allowing efficient pollen deposition. Similarly, this tactile surface, which resembles the typical conical cells of petals, may also act in pollinator attraction (Hansen et al., 2012; Costa et al., 2016).

On the other hand, the plain flowers of *Pseudotrimezia* show a more restricted range of visitors and higher ecological specialization than the ornamented flowers in the tribe. Often bees and hoverflies were the sole visitors, and thus the only putative pollinators. Furthermore, plain flowers bloom for a short period in the afternoon, mostly during the final hours of daylight, and offer pollen as the sole resource to putative pollinators. Pollen flowers are often less effective in attracting pollinator guilds than those that can offer additional resources (Willmer, 2011). Furthermore, pollinators were observed to fly directly to the central structure that combines the anthers and stigma in these flowers, where the pollen resource is offered (Fig. 6B; Appendix S9). This direct route might allow efficient pollen transfer from one individual to the next, since pollen collection is active in plain flowers. On the other hand, visitors of ornamented flowers may contact pollen passively while foraging for oil and/or nectar.

Plain flowers, which are radially symmetrical with a prominent central column formed by the style and anthers, are widespread
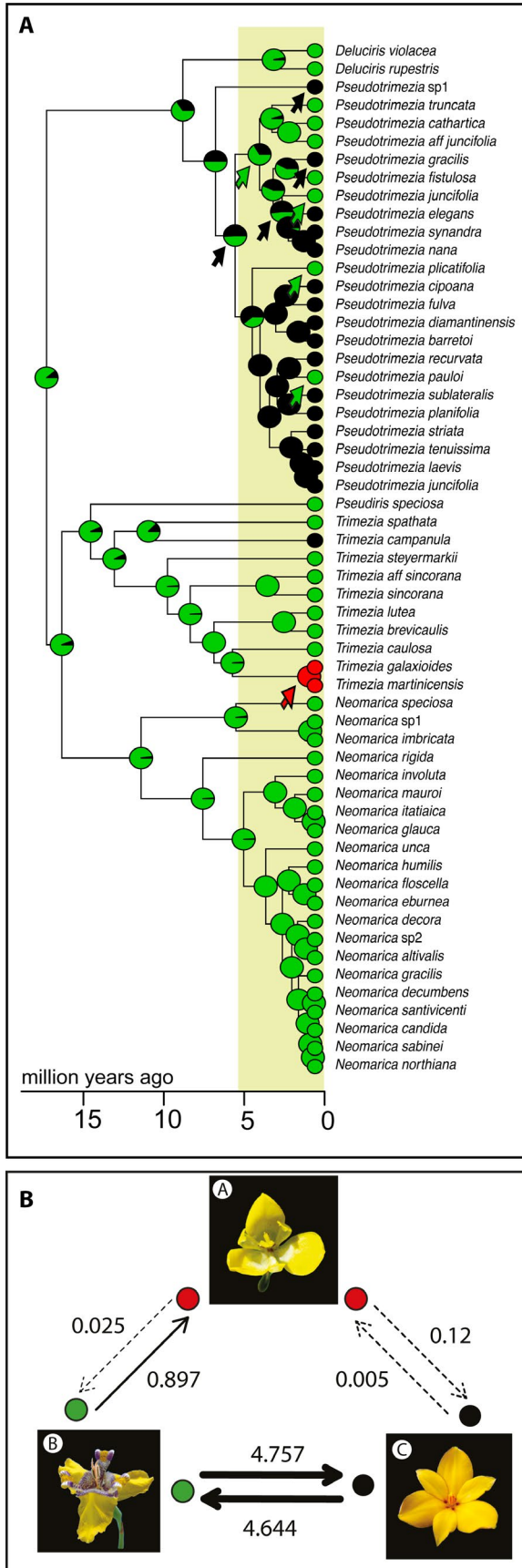
flowers of *P. juncifolia* and *P. truncata*, which brush the elaiophores with the oil-collecting apparatus of their forelegs. Oil production in similarly ornamented flowers has already been recorded in a few species of *Trimezia* and *Neomarica* (Vogel, 1974; Buchmann, 1987; Rudall et al., 2003; Simpson and Neff, 2006; Chauveau et al., 2012) and also in some species of the sister tribe Tigridieae: *Cypella* Herb., *Herbertia* Sweet, and *Kellisa* Ravenna (Chauveau et al., 2012; Oleques et al., 2020). Large display and production of nectar and oil are expensive traits in terms of energy, though they seem to attract several different pollinator guilds, at least in the case of the ornamented flowers of Trimezieae.

FIGURE 7. (A) Ancestral trait reconstruction of different floral displays on a phylogeny of Trimezieae tree based on combined nrITS + chloroplast data (from Lovo et al., 2018). Arrows of different colors show shifts in flower type (blackarrows: ornamented flowers; red arrow: intermediate flowers; green arrows: plain flowers). (B) Average number of shifts resulting between each floral development mode inferred from 1000 simulations of stochastic trait reconstruction. Note that average numbers of transitions between plain and ornamented flowers recovered are slightly different from those highlighted by arrows in panel A. This is expected, given that panel A is a summary from multiple simulations, and changes along branches are also allowed.

across the family (Goldblatt et al., 2008). This arrangement with stamens in close proximity to the stigma could also facilitate self-pollination (Goldblatt, 1981; Goldblatt et al., 2005). In fact, Goldblatt and Manning (2006) suggested that smaller, unscented flowers could be facultatively autogamous. Thus, we speculate whether the plain flowers of *Pseudotrimezia* might be facultatively autogamous or might exhibit mechanisms to avoid self-pollination, such as self-incompatibility, present in some species of *Moraea* (Goldblatt, 1981; Goldblatt et al., 2005). In summary, despite investing more energy in display and resources, the ornamented flowers of Trimezieae present a broader visitor spectrum compared with the plain flowers. Therefore, they fit to a more generalist strategy than the plain flowers regarding their pollination systems. This is a striking result and an unusual consequence of floral heterochonic processes, previously documented only for *Dalechampia* (Armbruster et al., 2012)

Linking developmental lability and diversification in Trimezieae

In addition to having a dramatic impact on the pollination strategy, heterochrony can promote relative lability in the evolution of Trimezieae. We observed at least eight shifts between the two extremes of the floral developmental spectrum observed in *Pseudotrimezia* within a short time frame of 5 myr (Fig. 7A). Moreover, these shifts appear to have occurred in the absence of an intermediate phenotype. We cannot rule out a possible masking of intermediates by extinctions, but this alternative seems unlikely given the recent evolution of this group. Pollination shifts are important drivers of diversification in the family Iridaceae (Goldblatt and Manning, 2006; Chauveau et al., 2012; Valente et al., 2012), and similar on-off patterns are observed elsewhere in the family (e.g., in *Homeria*, now sunk in *Moraea*; Goldblatt, 1998). Given that contrasting floral types attract different pollinator guilds and promote different pollinator behavior, heterochronic events here could lead to rapid reproductive isolation, contributing to increased speciation (e.g., Bateman et al., 2012).

In turn, this factor could have contributed to the high diversification rates observed in the different lineages within Trimezieae that are restricted to the *campos rupestres* floras (Vasconcelos et al., 2020). This putative link between floral specialization and geographic distribution merits further investigation. All of the ~20 plain-flowered species of Trimezieae are geographically restricted and endemic to a relatively small biogeographic region, the *campo rupestre* flora of the Southern Espinhaço Province (Colli-Silva et al., 2019). Two possible explanations can be drawn for this apparent correlation between restricted geographic distribution and higher specialization in pollination strategies: (1) These species could be geographically restricted because their pollinators are

found only in that area, as observed among montane species of *Mimulus* (Schemske and Bradshaw, 1999). The community of pollinators could affect plant distributions, restricting their dispersal and affecting local floristic composition (Sargent and Ackerly, 2008; Alcantara et al., 2014). However, the floral visitors and potential pollinator species of the plain-flowered plants are widely distributed in the Neotropics (Aguiar, 2012). (2) Other factors, such as environmental limitations of some specific/fundamental abiotic resources (Caruso et al., 2019), might result in a higher cost of producing expensive structures such as ornamented flowers with nectar and oils. Oligotrophic soils, high daily temperature oscillation, and wind exposure, which are common in the *campo rupestre* (Giulietti et al., 1997; Oliveira et al., 2016), could indirectly favor the evolution of less costly plain flowers. A low-cost system could be advantageous if it increases pollination chances and diminishes competition/interference, as usually inferred for specialized strategies such as that observed in the plain flowers. These flowers also occupy a restricted niche in time, as their anthesis is highly synchronized and restricted to a few hours, at a time of day when sympatric species from other families are running out of resources (Table 1; J. Lovo, personal field observation), as are other sympatric Iridaceae species of the ornamented flower type that typically lasts longer and finish anthesis earlier in the day.

In general, organisms invest more energy to be specialist than generalist, and extreme specializations are sometimes considered evolutionary dead ends (Barrett, 2013). However, in angiosperms, this extra cost becomes advantageous if it guarantees more efficiency in pollination. The scenario presented by *Pseudotrimezia* appears to represent a slightly different strategy, because the more specialist the flower, the less energy it spends, both in resources and in floral display. Interestingly, the opposite pattern of repeated generalization from pollen-pollinated flowers has been observed at a larger taxonomic scale in Melastomataceae (see Gavrutenko et al., 2020). Pollen-pollinated lineages of Melastomataceae, such as *Microlicia* and *Cambessedessia*, are particularly diverse and endemic to the *campo rupestre* (Bochorny et al., 2019; Versiane, 2019). In addition, many plant species from several families that display variable degrees of specialization to pollen bees have recently been described in this habitat (Mesquita-Neto et al., 2018; Nogueira et al., 2018; Konzmann et al., 2020). Some of these lineages also show an increased diversification in the *campos rupestres*, particularly *Chamaecrista* and *Microlicia* (Vasconcelos et al., 2020). Thus, we wonder whether specialized pollination by pollen bees could increase the persistence of lineages within this habitat, since abiotic resources and pollinator guilds are largely variable in time and space. We hypothesize that pollination by pollen-collecting bees has been crucial for the evolution of the *campos rupestres* flora; however, this hypothesis needs further investigation. Future studies, including gene flow, phenology, pollination network dynamics, and seed production analyses are necessary to better evaluate these ideas.

CONCLUSIONS

In summary, our study indicates that heterochronic processes may have led to repeated evolutionary shifts in pollination biology of Trimezieae, allowing the lineage to rapidly explore new ecological opportunities. Multiple shifts between extremes in the floral developmental spectrum have occurred in a short timescale, likely as a result of paedomorphosis, which helps explain the evolutionary lability of floral morphologies in the group. Surprisingly, transitions resulting in less elaborated and less costly flower morphology led to flower morphologies that are highly specialized to pollen-collecting bees. The multidisciplinary approach we use here shows that data from different fields can be integrated and investigated to improve our understanding of the origin and maintenance of biodiversity.

AUTHOR CONTRIBUTIONS

J.L. and S.A. conceived the study. J.L. performed the field and lab experiments, with the collaboration of M.G.S., P.J.R. and G.P. A.J.C.A. performed field observations and bee identifications. J.L. and T.N.C.V. performed the data analyses. J.L., S.A., and T.N.C.V. cowrote the manuscript, with input from all the authors.

DATA AVAILABILITY STATEMENT

Voucher information is available in the supporting information (Appendices S1, S4, and S5). All alignments (https://doi.org/10.6084/m9.figshare.13522883.v1), phylogenetic trees (https://doi.org/10.6084/m9.figshare.13522826.v1), and the trait matrix (https://doi.org/10.6084/m9.figshare.13522685.v1) used in this study are available at FigShare in the provided links.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Taxa with vouchers collected for each approach (scanning electron microscope, floral biology) and respective coding for ontogeny type.

APPENDIX S2. Flower development of an ornamented-type flower of *Neomarica speciosa* (Liebm.) A.Gil & Bittrich.

APPENDIX S3. Mature flowers of the three types of flower morphology in Trimezieae.

APPENDIX S4. List of visitors and respective plant populations.

APPENDIX S5. Locality of plant species populations.

APPENDIX S6. Maximum credibility tree of Trimezieae based on a combined data set with posterior probability values.

APPENDIX S7. Trait reconstruction results on the ITS data set.

APPENDIX S8. Trait reconstruction results on the chloroplast data set.

APPENDIX S9. Flower visitors and pollination behavior of pollen collecting in plain flowers of *Pseudotrimezia* R.C.Foster.

APPENDIX S10. Flower visitors and pollination behavior in ornamented flowers of *Pseudotrimezia juncifolia* (Klatt) Lovo & A.Gil.

APPENDIX S11. Flower visitors and pollination behavior in ornamented flowers of *Pseudotrimezia truncata* (Ravenna) Lovo & A.Gil.

APPENDIX S12. Video: *Pseudotrimezia juncifolia* (Klatt) Lovo & A.Gil flowers and visitor behavior: the hinge mechanism.

APPENDIX S13. Video: *Pseudotrimezia truncata* (Ravenna) Lovo & A.Gil flowers and visitor behavior: the hinge mechanism.

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